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Brief Report: Type I interferon causes a thrombotic microangiopathy by a dose-dependent toxic effect on the microvasculature

Abstract

Many drugs have been reported to cause thrombotic microangiopathy (TMA), yet the quality of evidence supporting a causal association is often weak. In particular TMA has been reported in association with recombinant type I interferon therapies, with recent concern regarding the use of interferon in multiple sclerosis patients. However a causal association has yet to be demonstrated.

Here we adopt a combined clinical, epidemiological and experimental approach to provide evidence of a causal association between type I interferon and TMA. First we show the clinical phenotype of cases referred to a national centre is uniformly consistent with a direct drug-induced TMA, with no plausible confounding factors. Second we analyse both national and global drug safety data and show that type I interferon-associated TMA is dose-dependent. Third, we show that dose-dependent microvascular disease is seen in a novel transgenic mouse model of interferon toxicity. This microvascular pathology includes specific pathological changes seen in patient biopsies and is dependent on activation of the interferon response through the type I interferon receptor (IFNAR).

Together our clinical and experimental findings reveal a causal link between type 1 interferon and thrombotic microangiopathy. As such, recombinant type I interferon therapies should be stopped at the earliest stage in patients who develop this complication, with implications for risk mitigation.

Key Points

- Type 1 interferon causes a dose-dependent thrombotic microangiopathy
- Recombinant type 1 interferon therapies should be stopped at the earliest opportunity in patients who develop thrombotic microangiopathy

Introduction

Thrombotic microangiopathy (TMA) syndromes are characterised by endothelial dysfunction, microangiopathic haemolytic anaemia and microvascular ischaemia, with diverse aetiologies which include drugs^{1, 2}. Clinicians evaluating TMA patients must decide whether a particular drug is likely to have caused the disease. This difficult decision requires high quality evidence, and recent work has highlighted the difficulty of attributing a causal relationship¹. For the large majority of drugs, causality with TMA is inferred from a few case reports, without wider pharmacoepidemiological or biological analyses, and is therefore prone to major bias and confounding¹.

This problem is exemplified by recombinant type I interferon therapies. Case reports have linked TMA to both interferon- α and interferon- β , the main subclasses of type I interferon (Supplementary Figure 1). Particular concern has been recently raised regarding interferon- β use in multiple sclerosis patients where fatal cases of TMA have been observed^{3, 4}. However a causal role for interferon remains to be demonstrated, and alternative confounding aetiologies such as other drugs, complement mutations and *E.Coli* exposure have been proposed⁵.

Given the current need to identify stronger evidence of a causal association with type I interferon, particularly in multiple sclerosis patients, we performed a detailed analysis of interferon-associated TMA cases presenting to a national TMA centre. We then analysed national and global drug safety data, together with a novel transgenic model of type I interferon toxicity.

Results and Discussion

The clinical phenotype of both new and index³ cases of type I interferon-associated TMA in multiple sclerosis patients was reviewed by the UK national thrombotic microangiopathy centre (n=8, Figure 1). All patients developed renal failure and involvement of at least one other organ, in particular brain and heart. (Figure 1A-D). No other confounding TMA triggers or anti-drug antibodies were identified^{1,6,7,8}. Interferon bioactivity was detected in renal biopsies (Figure 1E). The final diagnostic evaluation by the national centre was uniformly consistent with a chronic drug induced thrombotic microangiopathy^{9, 10}. To evaluate a direct association with the drug, we examined the relationship between TMA and interferon dose. Affected patients, who were all in the bottom weight quartile¹¹, received a significantly higher weight-adjusted dose than those who did not develop the complication (Figure 1G $P<0.001$ t-test). Dose-dependence was also observed across both UK and international drug safety data (Figure 1H, Figure 1I, $P=0.006$). Furthermore, an association between TMA and human states of elevated endogenous type I interferon was identified through an analysis of “interferonopathic” diseases (Supplementary Figure 1). Taken together these data suggest that interferon causes a direct, dose-dependent thrombotic microangiopathy.

Type I interferons (α and β) activate a downstream response through the common interferon- α/β receptor IFNAR¹² (Figure 2A). We therefore next asked whether the microangiopathy was caused by a direct toxic effect of the recombinant protein itself on small blood vessels. We first reviewed available preclinical toxicity studies for recombinant type 1 interferon therapies^{13, 14}. To date, microvascular toxicity has not been observed in extensive preclinical studies of human type I interferon in rodents^{13, 14} (Supplementary Figure 2). However we found that human interferon exerts no downstream effect on mouse endothelial cells (Figure 2A). In contrast a strong downstream interferon response was observed in the same endothelial cells with species-matched interferon (Figure 2B and Supplementary Figure 2). This strict species-specificity of signalling between type I interferon and IFNAR therefore limits interpretation of existing preclinical experimental rodent studies.

To overcome this problem we utilised a species-matched transgenic mouse model of type 1 interferon toxicity to evaluate the effects of chronic type 1 interferon exposure on small blood vessels. In our model, type 1 interferon is transgenically produced in the brain at zero (*WT*), low (*IFN^{Low}*) or high (*IFN^{High}*) level, leading to focal graded activation of the interferon response via IFNAR (Figure 2C)¹⁵. We analysed the microvasculature of these mice with both scanning electron microscopy of microvascular casts and quantitative pathology¹⁶. Using this approach we observed a spectrum of small vessel disease associated with local production of type 1 interferon (Figure 2D,E, Supplementary Figure 3). This type I interferon-associated microangiopathy was dose-dependent (Figure 2H $P < 0.001$ one-way ANOVA), and included pathological microvascular abnormalities seen in the biopsies of the patients described above, such as endothelial hyperplasia, luminal occlusion and microaneurysm formation (Figure 2F-G, Supplementary Figure 4). To confirm this pathology is caused by activation of the interferon response through the type I interferon receptor, we showed that both upregulation of interferon response genes (IRG) and microvascular disease were absent in the brain of *IFN^{High}* mice that were null for the type I interferon receptor (*IFN^{High} x IFNAR1^{-/-}* mice, Figure 2I,J)¹⁷.

To date, strong evidence for a causal association between type I interferon and thrombotic microangiopathy has been lacking. Our findings from converging lines of investigation indicate that type I interferon causes thrombotic microangiopathy, through a direct toxic effect on the microvasculature, fulfilling the Bradford Hill criteria for causation (Supplementary Table 3).

These findings have two implications relevant to the current safety concerns regarding type I interferon use in multiple sclerosis patients³. Firstly, the dose-dependence helps identify an at-risk patient group treated with high dose interferon. We have retrospectively and prospectively observed an incidence of TMA of approximately 1:1000 patient-years in populations treated with high dose-interferon in the UK, about 20-fold higher than current risk estimates⁵ (Supplementary Table 1). Patients of low weight (and hence higher relative dose) may be at even higher risk. Secondly, our findings highlight the need to stop the drug at the earliest stage of the complication. In our group of patients, interferon-induced TMA evolves over approximately 3 months, with a detectable prodrome (Supplementary Table 2). Despite this window of opportunity for early recognition, all patients described here presented at a late stage, *in extremis*, to emergency or critical care facilities, often after permanent organ damage has been sustained. These findings present a rationale for targeted monitoring for patients receiving high dose type I interferon therapy. This approach has recently been adopted as a standard of care for multiple sclerosis patients across Scotland, and has led to prompt cessation of type I interferon administration following early warning signs such as severe hypertension and renal dysfunction. Since the implementation of monitoring, no patients have developed fulminant organ failure due to interferon-associated TMA (Supplementary Figure 5).

Taken together, our findings show that type I interferon causes TMA. Clinicians should be aware of this strong association when assessing patients. Recombinant type I interferon therapies should therefore be stopped at the earliest stage in patients who develop TMA and monitoring of high risk patients considered.

Study Design

Additional methods are available in the Supplementary Appendix

Patients

Patients who developed thrombotic microangiopathy were referred to the national thrombotic microangiopathy centre in Newcastle for further evaluation. The study was approved by Newcastle and North Tyneside 1 Research Ethics Committee (MREC/1/3/83). Requests for safety data to the manufacturer and drug regulatory agencies were submitted in the context of a registered patient safety audit (NHS Lothian DCNQIT417).

Statistics

Reporting rate and rate ratios were determined using Stata version 13. 95% confidence intervals were determined according to an exact Poisson method (StataCorp.2013)

Transgenic mouse experiments

Animal studies were approved by University of Sydney Animal Ethics Committee. Transgenic mice with astrocyte-targeted brain-specific production of type 1 interferon (GFAP-IFN α 1) were generated as previously described¹⁵. The microvasculature was examined and quantified in brain from mice of two independent lines with either low (*IFN^{Low}*) or high (*IFN^{High}*) levels of transgene-derived IFN production. For controls, brain microvasculature was also examined from age-matched non-transgenic (wildtype) littermates. Scanning electron microscopy of microvascular casts of wildtype and *IFN^{High}* mice (n=6) was performed as previously described¹⁶. The total number of microvascular abnormalities was counted across three X20 magnification fields per anatomical region (cerebellum, cortex, brainstem, thalamus, hippocampus), with counting performed blind to genotype. Rescue experiments were performed by crossing *IFN^{High}* mice to *IFNAR^{-/-}* mice, which lack a functional type 1 interferon receptor¹⁷. One-way ANOVA and t-test was used to compare between 3 and 2 groups respectively (GraphPad Prism Version 6.0d).

Interferon response gene expression

BEnd.5 mouse endothelial cells were plated at 5×10^5 cells per well of a 6 well plate. Recombinant mouse or human IFN- α or IFN- β (R+D Systems) was added to culture media for 24 hours. RNA was extracted with RNeasy kit (Qiagen) and interferon response gene expression was measured by RT-PCR. For RNA from brain, ribonuclease protection assays (RPAs) for interferon response genes were performed and analyzed as described previously¹⁸.

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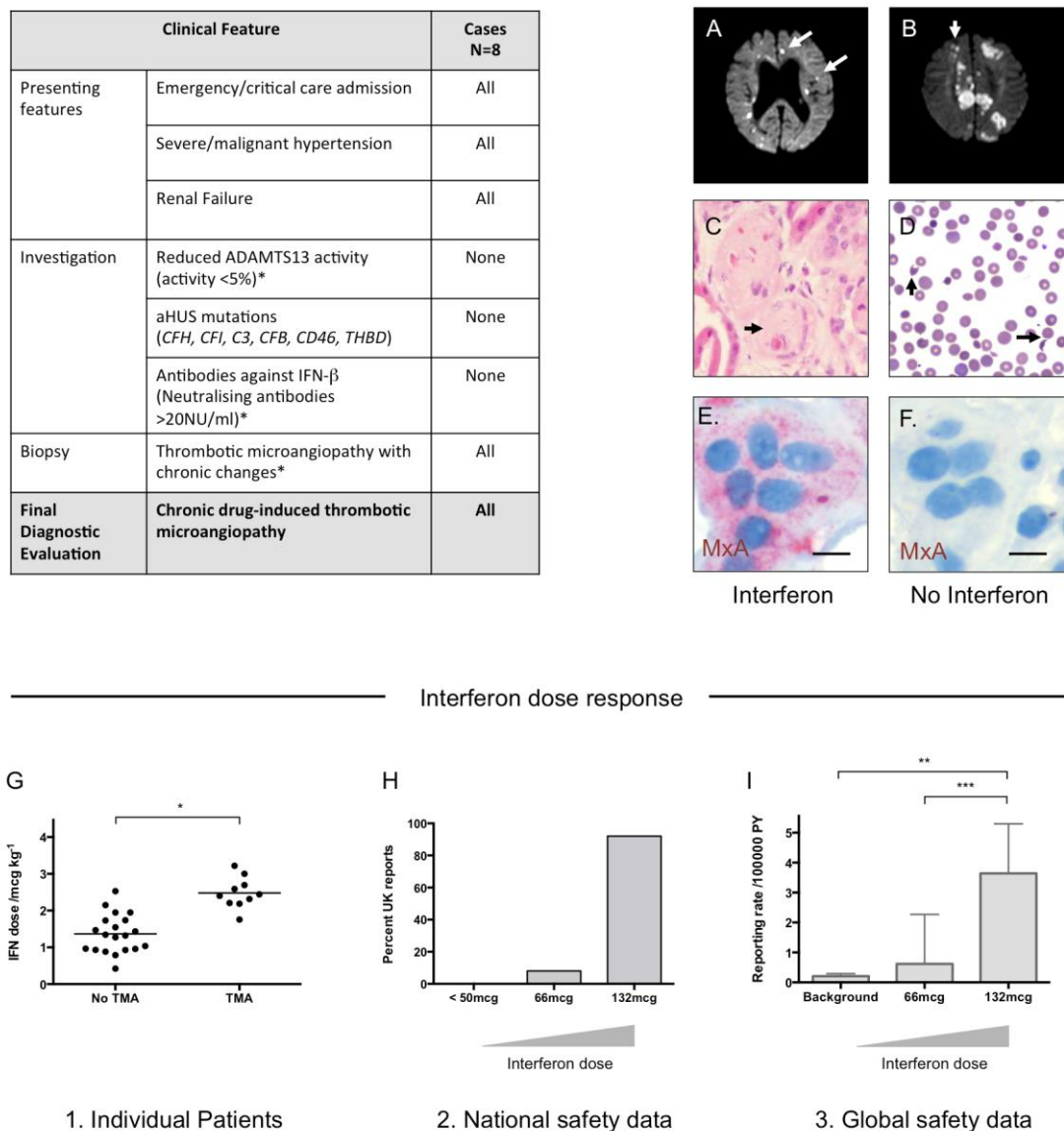


Figure 1. Recombinant interferon causes a direct dose-related thrombotic microangiopathy in multiple sclerosis patients

Summary of clinical features of new and index cases³ of interferon-associated thrombotic microangiopathy in multiple sclerosis patients referred to the national TMA centre in Newcastle. *= data available for 7/8 cases. Following diagnostic re-evaluation the clinical phenotype of the cases was uniformly consistent with a drug-induced thrombotic microangiopathy^{2, 9}. **(A,B)** Patient MRI brain scan shows multiple small new lesions consistent with recent ischaemia, identified on diffusion-weighted sequences (white arrows). **(C)** Haematoxylin and Eosin stain of renal biopsy from patient shows pathological microvascular changes with mucoid intimal thickening, luminal narrowing and trapped red blood cells (black arrow). **(D)** Admission blood film showing fragmented red blood cells (black arrows). **(E,F)** Evidence of activation of interferon response in renal biopsy of affected patient (MxA immunohistochemistry, red, scale bar 10 μ m), with comparable biopsy from patient with TMA not associated with interferon. **(G)** Patients who developed TMA received a higher weight-adjusted dose than unaffected MS patients treated with the same interferon- β preparation, * $P < 0.001$ t-test **(H)** All UK reports of thrombotic microangiopathy associated with interferon- β are associated with IFN β 1a dose >50mcg/week (n=14 reports) **(I)** Global reporting rates of thrombotic microangiopathy associated with IFN β 1a are dose-dependent (Data represent reporting rate with 95%CI, *** $P=0.006$, ** $P<0.0001$. Rate ratio 132mcg/wk to 66mcg/wk = 5.3 95%CI 1.3-46.2). Background rates of thrombotic microangiopathy in the UK population are shown ¹⁹.

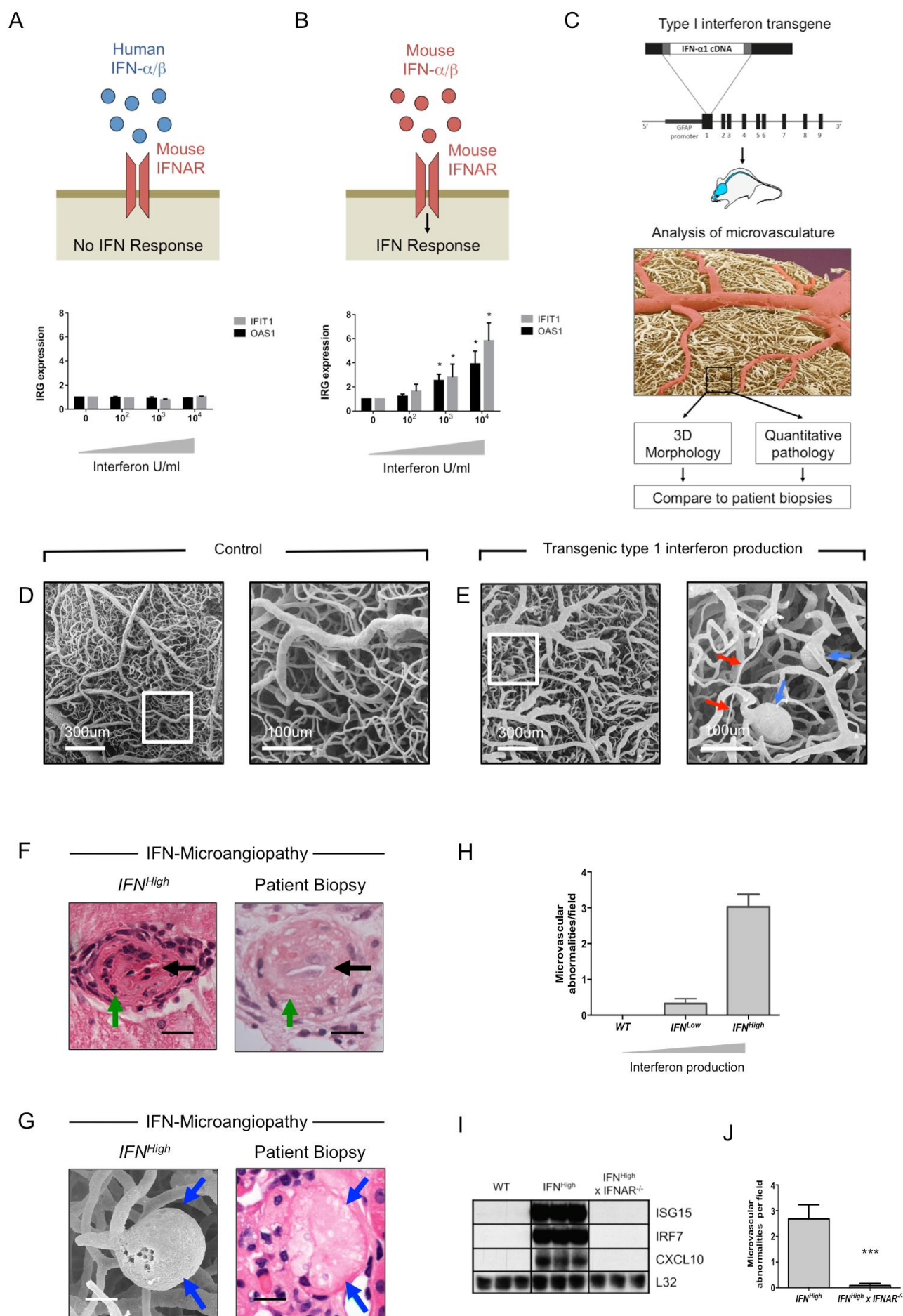


Figure 2. Type 1 interferon causes a dose-dependent microangiopathy

Type 1 interferon proteins ($-\alpha$ and $-\beta$) act through a common receptor (IFNAR), leading to activation of downstream interferon response genes (IRGs). **(A)** Preclinical toxicity testing of human recombinant type 1 interferon utilises cross-species testing. Human recombinant type 1 interferon does not elicit a downstream interferon response in mouse endothelial cells, as measured by quantitative PCR for type 1 interferon response genes in bEnd.5 brain endothelial cell line. **(B)** In contrast, dose-dependent upregulation of interferon response genes is observed in mouse endothelial cells exposed to same-species recombinant type 1 interferon *in vitro* (data represent mean \pm SEM * = $P < 0.05$, t-test compared to no interferon, $n=3$ experiments, IFN- α shown). **(C)** Overview of transgenic experimental design (see text), with scanning EM image of *IFN^{High}* vascular cast, coloured to show larger vessels (red) and microvasculature (box, yellow) **(D)** Scanning electron microscopy of a cerebrovascular cast of small blood vessels from control mouse showing normal microvascular architecture. **(E)** Abnormal morphology of brain microvasculature with variations in microvessel calibre (red arrows) and microaneurysms (blue arrows) is observed in all mice with highest levels of brain-restricted type 1 interferon production (Representative EM shown, $n=6$). **(F-G)** Microvascular pathology identified in this model includes specific pathological abnormalities such as luminal narrowing (black arrows), endothelial hyperplasia (green arrows) and microaneurysm formation (blue arrows), which were seen in the biopsies of the patients described above. Scale bars = $20\mu\text{m}$. **(H)** Microvascular pathology in interferon-overexpressing mice is dose-dependent, *WT*= wildtype, no transgenic overexpression, *IFN^{Low}* = transgenic line with low interferon overexpression, *IFN^{High}* = transgenic line with high interferon overexpression ($n=8$ mice per group. Data represent mean \pm SEM one-way ANOVA $p < 0.0001$). **(I)** Upregulation of interferon response genes (ISG15, IRF7, CXCL10) was observed in *IFN^{High}* transgenic mice and absent in mice which lack the type I interferon receptor (*IFN^{High} x IFNAR^{-/-}*). Total RNA was extracted from the brain of wildtype and transgenic mice and analyzed by RPA and visualized by autoradiography ($n=3$ per group). **(J)** Microvascular pathology was rescued in *IFN^{High}* mice which lack the type 1 interferon receptor (*IFN^{High} x IFNAR^{-/-}*). Data represent mean \pm SEM *** $P < 0.001$, t-test compared to *WT* and *IFN^{High} x IFNAR^{-/-}* $n=8$ regions.

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